



## Correlation between populations of *Rhodnius* and presence of palm trees as risk factors for the emergence of Chagas disease in Amazon region, Brazil

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### ABSTRACT

Among the states that comprise the legally defined Amazon region of Brazil, Pará has presented the highest occurrences of acute cases of Chagas disease over the last two decades. These cases have been attributed to consumption of fruits from native palm trees. In surveys in rural and wild areas of the municipality of Oriximiná, Pará, triatomine fauna, their main ecotopes and the infection rate due to *Trypanosoma cruzi* were identified using active and passive search methods: manual capture and Noireau traps, respectively. A total of 582 ecotopes were surveyed using 1496 Noireau traps. Out of 442 specimens collected, 289 were identified as *Rhodnius robustus* and 153 as *Rhodnius pictipes*. The infection rate caused by *T. cruzi* was 17.4%. The food sources of the triatomines were found to be birds, hemolymph, horses, and rodents. The association between *R. robustus* and *inajá* palm trees (*Attalea marita*), which are abundant in rural areas, was confirmed. On the other hand, *R. pictipes* is found in several palm tree species, such as *inajá* (*A. marita*), *muçajá* (*Acrocomia aculeata*), *murumuru* (*Astrocaryum murumuru*) and *pataúá* (*Oenocarpus bataua*), and in bromeliads in wild areas. These occurrences of triatomine species in regions with or without *T. cruzi* infection, in the vicinity of the main settlement of the municipality, suggest that there is a need for entomological and epidemiological surveillance in this region.

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### 1. Introduction

The triatomine fauna of the Amazon region comprises around 27 species distributed into nine genera (Aguilar et al., 2007). In the Brazilian Amazon region, at least 16 species have been identified, and 10 of them are infected with *Trypanosoma cruzi* (Junqueira et al., 2005).

In the state of Pará, there are 15 wild species of triatomines (Lent and Wygodzinsky, 1979; Galvão et al., 2003). Prominent among these are *Rhodnius pictipes*, *Rhodnius robustus*, *Triatoma maculata* and *Panstrongylus geniculatus*, which have shown invasive behavior

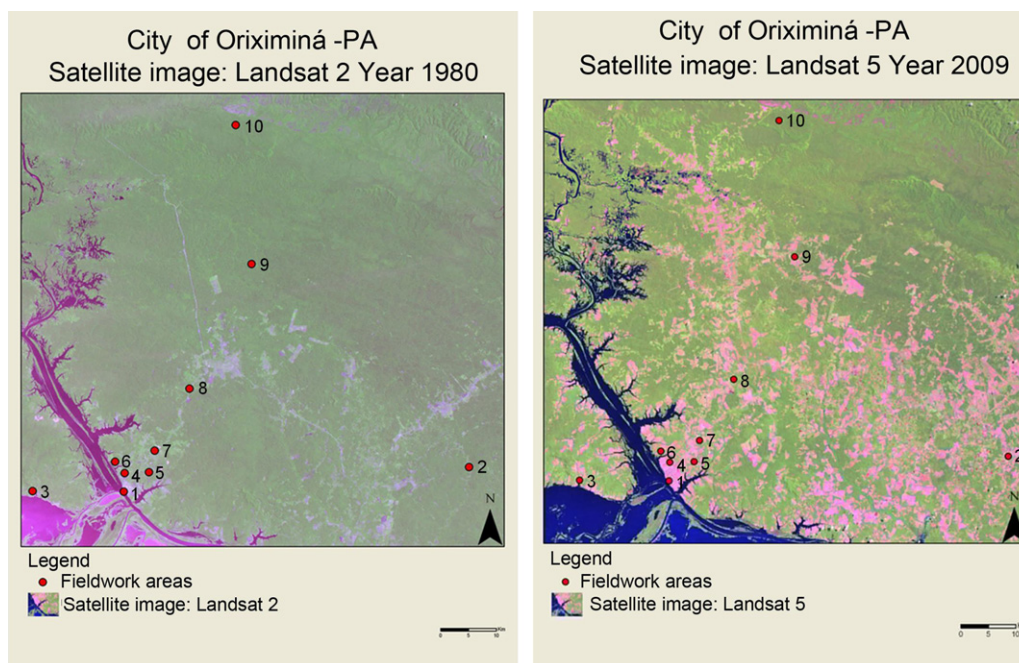
in the neighboring states (Miles et al., 1981, 1983; Valente et al., 1999; Luitgards-Moura et al., 2005).

In Neotropical regions, palm trees of the genus *Attalea* form well-defined ecotopes that act as important environments in which wild populations of *Rhodnius* species can form nests and find shelter (Mascarenhas, 1991; Romána, 2007; Dias et al., 2008). The *Attalea* complex encompasses four genera: *Attalea*, *Maximiliana*, *Orbignya*, and *Scheelea*. These are abundant and capable of forming large stands of trees in wild areas subject to human action.

The geographical distribution of wild species of *Rhodnius* overlaps the distribution of the palm trees, which occupy environments going from rainforest and dry forest to *cerrado* (savanna) (Dias et al., 2008; Lima et al., 2008). The degree of association between species of the genus *Rhodnius* and palm trees varies. Some species are more specialists, such as *R. brethesi*, which infests palm trees of the species *Leopoldina piassaba* (Mascarenhas, 1991), while others are more generalist, such as *R. pictipes*, which can be found in more than one genus of palm trees (Abad-Franch et al., 2005).

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**Fig. 1.** Advance of deforestation in the municipality of Oriximiná, state of Pará, Brazil, from 1980 to 2009, and the communities where specimens were caught. Geoprocessing Laboratory, LIS/ICICT. (1) Main urban center of Oriximiná, (2) Estrada do Laudica, (3) Sapucúá, (4) Paracuí, (5) Campina, (6) Caipuru, (7) Estrada do Lixão, (8) Poção, (9) BEC, and (10) Campos Gerais.

The presence of these plant species serves as an ecological indicator for areas that are at risk of *T. cruzi* transmission, since they provide shelter not only for the vectors but also for the hosts that participate in the wild transmission cycle of this flagellate protozoan (Romaña, 2007).

According to Aguilar et al. (2007), the risk that this disease may become established as a significant threat to the population of the Amazon region is related to a complex group of biological interactions and social determinants. These, which are related to increasing numbers of cases of Chagas disease, include human economic activity, which causes disorderly population growth in rural areas. Moreover, migration of human populations from endemic regions, often accompanied by domestic reservoirs of *T. cruzi* or its vectors, allows the introduction of non-autochthonous species that are infected with this protozoon. This provides the possibility for triatomines to become domesticated in areas where this had not previously been noted, thereby constituting a public health problem (Forattini, 1980).

In the eastern part of the Amazon region, the municipality of Oriximiná (Pará) is located on the left bank of the Trombetas river and borders Guyana and Suriname to the north and the states of Amazonas and Roraima to the west. In the border regions and at the boundary with the municipality of Santarém, cases of Chagas disease have already been reported (Rambajan, 1984; Oostburg et al., 2003; Pinto et al., 2008).

At the end of the 1970s, large companies began operations on the banks of the Trombetas river, for bauxite extraction, which generated migration of labor and consequently led to population growth in the municipality. Comparison of the vegetation coverage in the 1980s with the situation in 2009 (Fig. 1) shows that the region has been visibly altered through deforestation. The deforested areas are used not only for subsistence farming, but also as artificially seeded pasture and secondary forest (Ministério da Saúde, 2009). Palm trees of the genus *Attalea*, the preferred biotope of *Rhodnius*, have grown in these secondary forest areas (Romaña, 2007). Within this context, it can be suggested that the dynamics of parasite transmission within the wild cycle may have changed, thereby placing

humans in contact with the natural foci and possibly giving rise to establishment of the domestic cycle of Chagas infection (Barreto, 1967).

Hence, the triatomine fauna and its eco-biology will be investigated to know the risk of transmission of Chagas disease in this region.

## 2. Materials and methods

### 2.1. Study area

The municipality of Oriximiná (4°45'48"S and 55°22'09"W) comprises a frontier region located within the Amazon biome, in the northwest of the state of Pará and in the lower Amazon mesoregion (Fig. 2). The human population is about 61,125 inhabitants. The rainy season coincides with the months of December to June, which is characterized as the "winter" period, while the less rainy months, from July to November, is regarded as the "summer" period. The annual rainfall volume is irregular, but is around 2000 mm. The air temperature is always high, with an annual mean of 25.6°C (Ministério da Saúde, 2009).

The investigations were carried out at 25 georeferenced points, randomly chosen, distributed among nine communities, in the months of November 2007 and April, June, August, and November 2008, in rural and wild areas. The communities of Caipuru (40 inhabitants), Estrada ("Highway") do Laudica (40 inhabitants), Estrada do Lixão (80 inhabitants), and Paracuí (40 inhabitants) consist of rural areas given over to cattle rearing, with very few human dwellings; the community of Poção (60 inhabitants) includes both rural and wild areas; and the communities of BEC ("Batalhão de Engenharia e Construção") (560 inhabitants), Campos Gerais (50 inhabitants), Campina (80 inhabitants), and Sapucúá (350 inhabitants) consist of wild areas. Despite the higher numbers of inhabitants in the BEC and Sapucúá communities, the wild environment remains preserved.

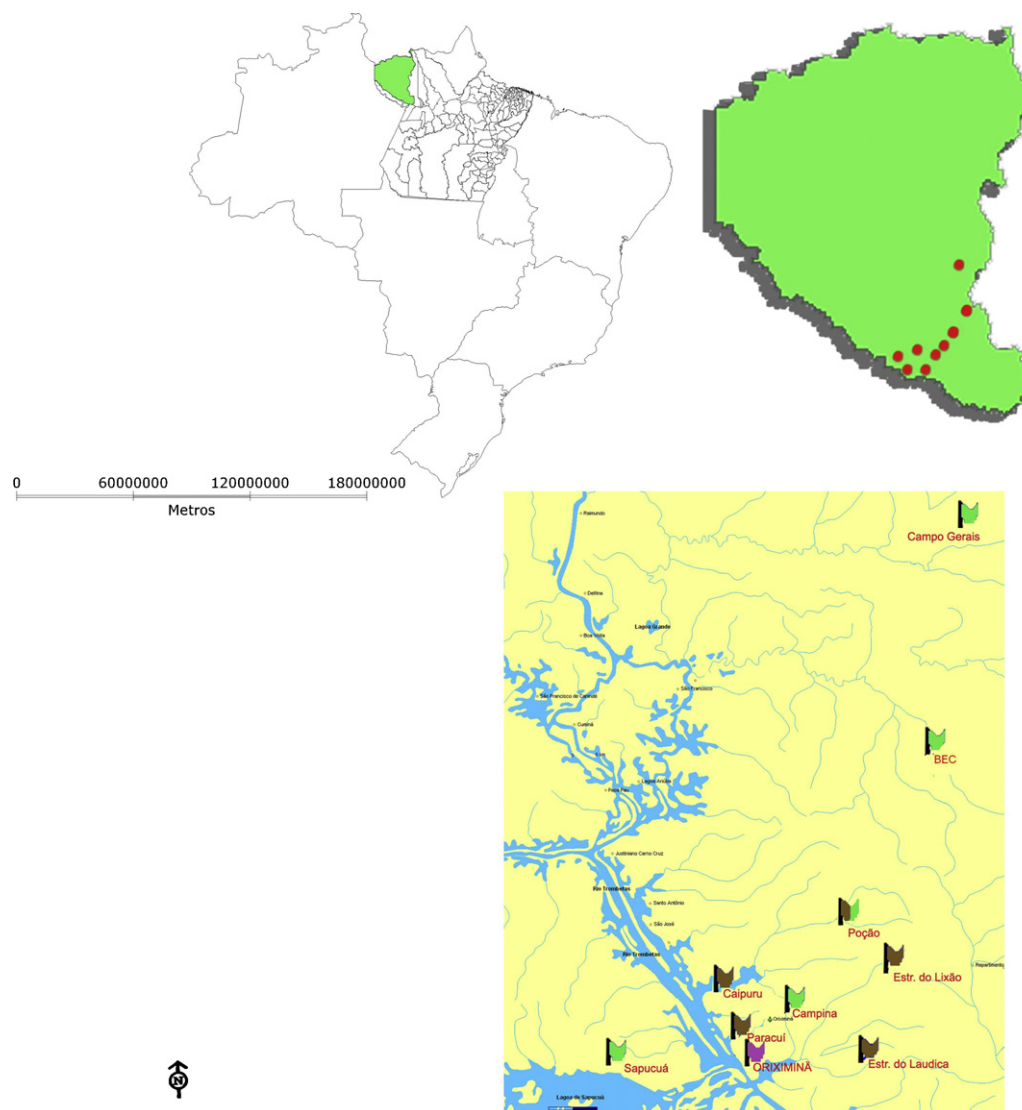


Fig. 2. Locations of the nine communities studied in the municipality of Oriximiná, state of Pará, Brazil.

## 2.2. Triatomine sampling

The searches were conducted actively, looking only under bark, bromeliads, rocks, and roots, with the aid of tweezers but without using dislodging devices, and passively, using traps as described by Noireau et al. (1999), with Plasto double-sided adhesive tape so that the insects would be retained better. In this case, the ecotopes investigated were palm trees (crown, spathe, and inflorescence) and associated vegetation, along with tree trunks, tree hollows, animal burrows, animal nests, and rock outcrops. The palm trees were identified using the dichotomous key of Miranda and Rabelo (2006). The traps were left out from the afternoon to the next morning, thus totaling 12 h, on average.

## 2.3. *Trypanosoma cruzi* infection index

To investigate infection due to *T. cruzi*, feces and/or urine were obtained by means of abdominal compression and were seeded into NNN culture medium (Novy and McNeal, 1904; Nicole, 1908) plus 4 ml of liver infusion tryptose (LIT) (Camargo, 1964), and supplemented with 20% fetal bovine serum, in order to isolate the parasite. The dead specimens were dissected under sterile

conditions in order to remove the digestive tube, which was also seeded into culture medium (Gonçalves, 2000).

The molecular typing was done starting from around 50 ng of genomic DNA from the isolate, which was identified as Orix 1 (Oriximiná 1). The divergent domain D7 of the 24Sα rDNA gene was PDR amplified with the primers D71 and D72, as described by Souto et al. (1996). Genomic DNA from the clones Dm 28c (*T. cruzi* I) and Y (*T. cruzi* II), and the strains 3663 (ZIII-A) and 4167 (ZIII-B), was used as controls. The amplification products were run in 7.5% polyacrylamide gel and stained with ethidium bromide.

## 2.4. Blood meal source

To identify the food source, feces and urine obtained by means of abdominal compression were used, and also those that were impregnated on the filter paper of the flasks used to transport the insects. Out of the total of 54 samples analyzed, 39 were obtained by means of abdominal compression and 15 from the filter paper. The ELISA test was performed in accordance with the protocol described by Burkot et al. (1981), as modified by Duarte and Marzochi (1997). The samples were diluted in carbonate–bicarbonate buffer and applied to 96-well polystyrene microplates. After two hours of incubation, the antiserum was added. The samples then underwent

**Table 1**

Numbers of Noireau traps, ecotopes and triatomines captured in the communities investigated in the municipality of Oriximiná, state of Pará, Brazil.

Communities	Noireau traps		Ecotopes			Triatomines captured		
	Number	Positive (%)	Surveyed	Positive (%)	P value ( $\chi^2$ test) <sup>a</sup>	<i>R. robustus</i>	<i>R. pictipes</i>	Total
Caipuru	561	103 (18)	157	53 (34)	0.005	242	1	243
Campina	32	4 (12)	32	4 (12)	NS	0	9	9
Campos Gerais	199	2 (1)	62	2 (3)	NS	0	2	2
Estrada do BEC	37	2 (5)	37	2 (5)	NS	0	3	3
Estrada do Laudica	52	0 (0)	13	0 (0)	NS	0	0	0
Estrada do Lixão	51	2 (4)	22	1 (4)	NS	0	1	1
Paracuí	30	1 (3)	10	1 (10)	NS	0	1	1
Poção	382	75 (20)	145	46 (30)	NS	47	119	166
Sapucúá	152	8 (5)	107	8 (7)	NS	16	1	17
Total	1496	197 (13)	585	117 (20)		305	137	442

<sup>a</sup>  $\chi^2$  test (with Yates correction when necessary) to compare the no. of positive ecotopes and traps for communities. df = 1, NS, not significant.**Table 2**Positives ecotopes for *Rhodnius robustus* and *Rhodnius pictipes* captured in the municipality of Oriximiná, state of Pará, Brazil.

Ecotopes	Surveyed	Positive (%)	<i>R. robustus</i>	<i>R. pictipes</i>	Total
Inajá ( <i>Attalea marita</i> )	366	87 (74.2)	268	44	312
Bromeliads/Inajá <sup>a</sup>	25	12 (10.2)	15	13	28
Murumuru ( <i>Astrocaryum murumuru</i> )	24	5 (4.3)	5	28	33
Pataúá ( <i>Oenocarpus bataua</i> )	24	4 (4.3)	0	9	9
Bromeliads/Mucajá	16	6 (5.1)	0	22	22
Tucumã ( <i>Astrocaryum aculeatum</i> )	10	1 (0.9)	1	0	1
Abiorama ( <i>Lucuma lasiocarpa</i> )	3	1 (0.9)	0	8	8
Mucajá ( <i>Acrocomia aculeata</i> )	2	1 (0.9)	0	29	29
Total	470	117 (100)	289	153	442

<sup>a</sup> In these ecotopes mixed infestations were found, two inajá (*Attalea marita*) palms trees and one bromeliad inajá (*Attalea marita*) associated.

a second wash, the conjugate (horseradish peroxidase-conjugated anti-rabbit IgG) was added, and a new incubation and wash were performed. This was carried out with buffer application, and the plates were analyzed in a microplate reader with a 490 nm operational filter and a 630 nm reference filter (Burkot et al., 1981). The results were interpreted after the cutoff point had been established. This was defined as the mean value of the negative controls plus two standard deviations. Positive samples were those reading 10% over the cutoff. Each sample was tested using 11 antisera: bird (*Galus gallus*), goat (*Capra aegagrus*), horse (*Equus caballus*), dog (*Canis familiaris*), opossum (*Didelphis marsupialis*), cat (*Felis domesticus*), human (*Homo sapiens*), reptile (*Tupinambis merianae*), rodent (*Rattus norvegicus*), armadillo (*Dasypus novemcinctus*), and cockroach hemolymph (*Blattella germanica*).

### 2.5. Statistical analysis

The data were analyzed using the GraphPad InStat v.3.05 software (GraphPad Software, San Diego, CA, USA). Chi-square tests ( $\chi^2$ ) were used to compare distributions among several categories. Differences were considered significant for  $P < 0.05$ .

## 3. Results

The searches using active methods were negative, and thus differed from the results obtained through the passive search, in which, out of the 1496 traps used, 197 (13%) were positive for the presence of triatomines. Among the nine communities investigated, only Estrada do Laudica was negative. The differences in the numbers of positive ecotopes and traps for communities were not significant ( $\chi^2$ ;  $P > 0.05$ ) (Table 1).

In all, 24 types of ecotope were investigated, consisting of palm trees, bromeliads, and abiorama (*Pouteria caimito*). Among these ecotopes, eight were positive for the present of triatomines and 16 were negative (Tables 2 and 3). Among the palm trees, the following were prominent: inajá (*Attalea marita*), mucajá

(*Acrocomia aculeata*), murumuru (*Astrocaryum murumuru*), tucumã (*Astrocaryum aculeatum*) and pataúá (*Oenocarpus bataua*). With regard to associated vegetation, triatomines were found in epiphytic bromeliads growing on mucajá and inajá palm trees, and on abiorama trees. This last case was a more eclectic occurrence, i.e., not restricted solely to palm trees (Table 2).

Out of the total number of 585 ecotopes investigated, 318 were located in wild areas and 267 in rural areas. The presence of triatomines was signaled in 117 ecotopes, which resulted capture of 442 specimens: 289 *R. robustus* and 153 *R. pictipes* (Table 2).

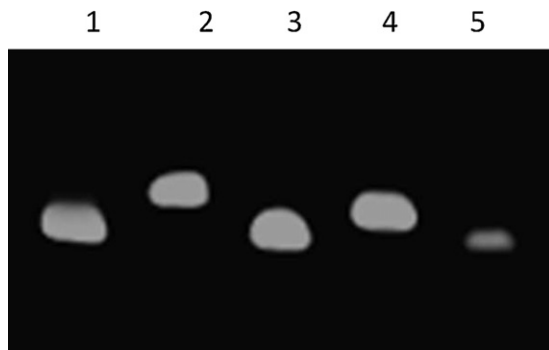
In the inajá palm trees, which were commonest type, there was a significant difference (chi-square approx = 16.58, df = 1,  $P < 0.0001$ )

**Table 3**

Negative ecotopes for triatomines, in the municipality of Oriximiná, state of Pará, Brazil.

Negative ecotopes	Number of ecotopes	
	Surveyed	Traps used
Palm trees		
Açaí ( <i>Euterpe oleracea</i> )	27	28
Bacaba ( <i>Oenocarpus bacaba</i> )	9	17
Buriti ( <i>Mauritia flexuosa</i> )	5	13
Mumbaca ( <i>Astrocaryum gynacathum</i> )	1	1
Pajurá ( <i>Couepia bracteosa</i> )	4	4
Others		
Abacatirana ( <i>Ocotea costulata</i> )	1	1
Apuizeiro ( <i>Ficus</i> sp.)	3	3
Hole in the ground	5	5
Castanheira ( <i>Lecythis pisonis</i> )	3	4
Tree cavities	19	19
Rocks	19	52
Root	1	2
Under bark	1	1
Taperebá ( <i>Spondias mombin</i> )	1	1
Timborana ( <i>Piptadenia suaveolens</i> )	1	1
Burrows	10	19
Bark	5	5
Total	115	176





**Fig. 3.** Molecular characterization of the isolate Orix 1, from analysis on the divergent domain D7 of the 24Sα rDNA gene using electrophoresis on 7.5% polyacrylamide gel stained with ethidium bromide. (1) Dm 28c (*T. cruzi* I); (2) Y (*T. cruzi* II); (3) strain 3663 (ZIII-A); (4) strain 4167 (ZIII-B) (samples 1–4 were used as controls); and (5) Orix 1.

between the numbers of *R. robustus* and *R. pictipes* that were caught. For the other ecotopes, there was no significant difference (not showed data). In the *abiorama* trees, the bromeliads associated with *mucajá* palm trees and the *mucajá* and *pataúá* palm trees, *R. pictipes* was observed. On the other hand, in *tucumã* palm trees, only *R. robustus* was observed. In three *inajá* palm trees and in one epiphyte bromeliad on an *inajá* palm tree, the two species were found concomitantly (Table 2).

In the wild environments, seven ecotopes (6%) were positive for *R. robustus* and 36 (30%) for *R. pictipes*, while in the rural environments, 68 ecotopes (57%) were infested with *R. robustus* and eight (7%) with *R. pictipes*. Regarding the numbers of triatomines captured, 21 *R. robustus* and 136 *R. pictipes* were found in the wild environments and 268 *R. robustus* and 17 *R. pictipes* in the rural environments. The difference in the numbers of ecotopes infested between the wild and rural environments, and the triatomine species associated, was significant ( $P < 0.0001$ ).

Out of the 442 triatomines caught, 430 were nymphs and 12 were adults. Among the nymphs, 281 belonged to the species *R. robustus* and 149 to *R. pictipes*. Among the adults, eight were *R. robustus* and four were *R. pictipes*. Regarding the types of environment, in the forests, *R. robustus* only presented the nymph stages, while *R. pictipes* was represented by juvenile and adult forms. In the rural environments, the inverse was observed, i.e., *R. robustus* was found at all stages of development, while *R. pictipes* only presented the nymph stage.

The presence of infection due to *T. cruzi* was investigated in 72 specimens (38.9%). Of these, 30 were positive, thus representing a natural infection rate of 17.4%. One isolate was characterized as *T. cruzi* Z3-A corresponding to TcIII, in accordance with Zingales et al. (2009), and was identified as Orix 1 (Fig. 3).

The results obtained from analysis on the food sources showed that, out of the 54 triatomines examined using the ELISA test, 20 were positive. For *R. robustus*, the blood source that was most detected was birds, followed by horses and hemolymph. For *R. pictipes*, hemolymph was the main blood source followed by horses, rodents and birds.

#### 4. Discussion

Among the nine communities investigated, only in Estrada do Laudica were all the traps negative for the presence of triatomines and associated fauna. This community presented smaller numbers of palm trees and other ecotopes, which confirms the observation of Abad-Franch et al. (2010) that in intensely disturbed areas, colonies of triatomines tended to become rare.

The eclecticism of *R. robustus* and *R. pictipes* regarding natural ecotopes that was reported by Miles et al. (1983) and Abad-Franch et al. (2005) was only confirmed for *R. pictipes*, which was found in four species of palm tree (*inajá*, *mucajá*, *murumuru*, and *pataúá*) and in two types of associated vegetation (*abiorama* trees and bromeliads). For *R. robustus*, this characteristic was so striking, since it was mostly associated with *inajá* palm trees, and only in small numbers in *murumuru* and *tucumã* palm trees and in bromeliads in *inajá* palm trees.

The density of the *inajá* palm trees may have been influenced by several factors. Among these, the fragmentation of the forest in the central Amazon region can be cited. Here, it has been observed that human action directly influences the distribution of these palm trees (Scariot, 2001). These trees are resistant to deforestation and burning, and they survive in open areas and become dominant over other species, thus forming almost homogenous groups of palm trees (Miranda and Rabelo, 2006).

It is believed that the existence of groups of *inajá* palm trees in rural environments favors the growth of populations of *R. robustus*, thus making the numbers of these triatomines greater than in wild areas, where the density of these palm trees is low (Henderson and Scariot, 1993). Moreover, because these locations have been prepared for cattle rearing, humans not only establish themselves in this environment but also provide another source of food (Rojas et al., 2005).

Both triatomine species were found concomitantly in three *inajá* palm trees and one bromeliad. This confirms the affirmations of Miles et al. (1983) regarding the difficulty of distinguishing between the niches occupied by *R. pictipes* and *R. robustus*, given that both of them may occupy the same palm trees.

Among the negative ecotopes, the *acaí* and *bacaba* palm trees were highlighted. The architecture of these palm trees does not favor accumulation of organic material and the consequent growth of associated vegetation that would be necessary for forming a microhabitat suitable for sheltering hosts and reservoirs of *T. cruzi* and triatomines, and thus, colonization of these ecotopes (Lorenzo et al., 2003; Dias et al., 2008).

Consumption of *acaí* juice has been associated with cases of oral transmission of *T. cruzi* in the Amazon region (Valente et al., 1999; Valente, 2008). It has been speculated that the fruits may come from the field mixed with triatomines, or that the triatomines may be attracted by lights at the time of juice preparation, thereby contaminating the juice (Valente et al., 2009). In the present study, it was observed that at the time of palm fruit harvesting, the fruit picker who shinned up the tree to reach the fruit cluster left on the ground the basket or sack for packing the fruit, which would be taken to the fruit processors. Given the flexibility of the trunks of these palm trees, the action of climbing the tree would cause the crown to thrash against neighboring vegetation (other palm trees, associated vegetation or bromeliads), as if a sweeping action was being performed. It is believed that this movement would be sufficient to dislodge some triatomines from other, adjacent palm trees, which might fall into the above mentioned receptacles.

In the same way, edible palm fruits such as *pataúá*, *buriti*, and *bacaba* (Ferreira, 2005) may also be related to the oral transmission mechanism. Today, this is recognized as an important route generating morbidity and mortality through acute forms (Pérez-Gutiérrez et al., 2006). Another contamination route may be through the handling and removal of these fruits. Since the fruits are in the form of a cluster, they are very close to the trunk of the palm tree, where triatomines may be sheltering. Thus, during the handling to remove the fruits, care needs to be taken, given that if colonies of triatomines exist, the tools used may become contaminated.

Not only the palm trees *Attalea maripa* (*inajá*) (Miles et al., 1983; Romaña, 2007) and *Acrocomia aculeata* (*mucajá*) (Miles et al., 1983;

Diotaiuti and Dias, 1984; Dias et al., 2008) have been highlighted as epidemiologically important ecotopes presenting risk, but also *A. murumuru* (*murumuru*) and *O. bataua* (*pataúá*), which are common in the Amazon region and have been found to be infested with triatomines.

Identification of the feeding habits is an important information for understanding the epidemiology of Chagas disease. Out of the 54 samples, 34 did not react to any of the antisera tested, which suggests either that these specimens had been feeding on another vertebrate for which the antiserum was not tested, or that no stomach content was available (Dias et al., 2008; Lima et al., 2008) given that 97.29% of the specimens were first, second or third-stage nymphs and that they were in a state of fasting at the time of the examination.

Although material in the hindgut has been degraded, it still contains immunoglobulins. Since the immunoglobulin is species-specific, only the presence of these molecules ensures a reliable result in identifying the blood taken from a residual meal source (Gill, 1984). Thus, we chose to use ELISA because it is a sensitive and specific method that only requires small amounts of sample.

Feeding habits may be a reflection of the availability of food in the ecotopes, given that the habitat has a greater influence on the distribution of triatomines than does the food source (Miles et al., 1981). For some species, ornithophily may be an opportunistic form of behavior, rather than a selective characteristic (Diotaiuti and Dias, 1987), as described by Luitgards-Moura et al. (2005) for *T. maculata*, which was considered to be ornithophilic, but promptly adapted to feeding on *Mus musculus*. Thus, the palm tree densities provide appropriate conditions for *R. robustus* to consume bird's blood (Naiff et al., 1998), given that palm trees are places of refuge and nest construction for birds. The same opportunistic behavior may have occurred in relation to feeding from horses, which was found for *R. pictipes* and *R. robustus*, as also observed for *Triatoma vitticeps* (Gonçalves, 2000) and *Triatoma rubrofasciata* (Lorosa et al., 1998).

Hemolymph was the second commonest food source, which was found for both species, thus suggesting that in the natural environment, triatomines may resort to feeding from arthropods in the absence of vertebrates (Lorosa et al., 2000). However, *R. pictipes* demonstrated greater hemolymph consumption with scarcity of food sources in the ecotopes where it was caught.

The presence of infection due to *T. cruzi* demonstrated the existence of an enzootic cycle for the parasite in *R. robustus* caught in the community of Caipuru. The isolate Orix 1 was characterized as TcII, in accordance with other results from the Amazon region (Mendonça et al., 2002).

## 5. Conclusions

Because of the economic importance of the municipality of Oriximiná, state of Pará, Brazil, it has been expanding towards the wild environment, both in the bauxite extraction area and in the vicinity of the main settlement, which may favor contact between humans and vectors. Although there have not been any notifications of Chagas disease so far, the presence of triatomines in the periurban environment shows that it is important to implement entomological surveillance strategies and preventive strategies against Chagas disease in the municipality of Oriximiná.

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